## CONTRIBUTIONS TO THE BIOLOGY OF THE GREAT LAKES.

# THE BIOLOGICAL RELATION OF AQUATIC PLANTS TO THE SUBSTRATUM.

BY

## RAYMOND H. POND.

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### INTRODUCTION.

This investigation was undertaken at the suggestion of Prof. Jacob Reighard, in charge of the biological survey of the Great Lakes under the auspices of the United States Fish Commission. It was carried on during three years, chiefly in the summer, partly at Put-in Bay, Ohio, and partly at Ann Arbor, Mich., under the direction of Prof. F. C. Newcombe, of the University of Michigan, to whom I am indebted for constant guidance. To Mr. A. J. Pieters, of the U. S. Department of Agriculture, I am indebted for the use of his very complete bibliography of aquatic plants. The discussion of the papers by Forel, Hoppe-Seyler, Seligo, and Stockmayer, constituting the introduction as well as the larger portion of the chapter on economic significance of results, is from the pen of Prof. Jacob Reighard.

One of the objects of the biological survey of the Great Lakes was to ascertain the factors which determine the quantity of food fish it is possible for these lakes to support. To this end it was necessary to study not only the fishes themselves, but all forms of animal and plant life in the lakes, for upon these, directly or indirectly, the fishes depend.

That the larger aquatic plants play an important part in the biology of fresh water has been long recognized, and at least two rôles have been assigned to them. The first of these is mechanical. Often the plants growing submerged are so abundant as to cover the bottom. Their fine rootlets give to the bottom soil greater coherence, while their stems and leaves protect it from the mechanical action of the waves. Such plants, moreover, form aquatic meadows in whose dense growth multitudes of small animals and young fish find shelter and concealment from pursuing enemies. Some fishes select these meadows as localities in which to lay their eggs, and the minute plant and animal

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forms there present furnish a plenteous food supply for the young fish. Although the larger plants as such are, while living, little used as food by the aquatic animals, yet they greatly increase the surface available for the attachment of microscopic plant forms, which are eaten by the smaller animals, and the latter in their turn by the fishes. This relation of the larger plants to the food supply is, as Seligo (1890, pp. 46, 47) pointed out, chiefly mechanical and indirect.

The second rôle usually assigned to water plants is that of aeration, in which the plants by their carbon assimilation remove carbon dioxid from the water and give out oxygen in its place. Aquatic animals use the oxygen which is in solution in the water and give off carbon dioxid, which passes into the water, and which, if it should accumulate excessively, would become fatal to the animals. The water must, then, be constantly supplied with fresh oxygen and as constantly freed of the greater part of its carbon dioxid. In sunlight plants absorb carbon dioxid, and in using it for the manufacture of carbon compounds give off oxygen to the water in equal volume to the carbon dioxid absorbed, so that green plants during sunlight not only keep the proportion of carbon dioxid down, but actually become aerating agents by reason of their contributions of oxygen. Hence it has been the current belief that aquatic plants are necessary to furnish the oxygen needed by aquatic animals and to remove from the water the carbon dioxid injurious to the animals.

In 1890, however, Seligo indicated that the importance of the aeration rôle of aquatic plants has probably been exaggerated.

For, as is well known, plants need in their life processes not only the nourishing carbon dioxid, but like all other living things oxygen also, and while the excretion of oxygen takes place only in sufficient light, the absorption of oxygen goes on continuously. If then the oxygen content of water rich in plants must indeed be greater by day, so is it for the same reason much the less by night. At the same time equalization of gases must take place very rapidly in the comparatively shallow shore region of the lake basin, not only by access of the outer air, especially through wave motion, but also by diffusion within the water mass itself; and just as the assumption that forest air must be richer in oxygen than the air in the larger cities, for instance, has been shown by careful air analysis to be erroneous, so can the oxygen content of the shore water rich in plants be scarcely different from that which is free from plants. (Seligo, 1890, p. 47.)

Oxygenation of the superficial layers of water is accomplished by mechanical admixture of air through the action of waves, tributary streams, and rainfall, so that the upper 2 meters, over the entire surface of the lake, is practically saturated with the atmospheric gases. Oxygen thus absorbed from the air has been usually thought, as by Seligo, to diffuse with great rapidity into the deeper layers of the water, but Hoppe-Seyler (1896, p. 15) has measured the rate of diffusion of oxygen into motionless water from the atmospheric air and has found it extremely slow and wholly inadequate to account for the

relatively large volume of oxygen present in the deeper water of lakes (about 7.6 c. c., per liter of water). He thinks it probable that the migrations of animals from the superficial water toward the bottom and back again aid diffusion by mechanically mixing the water, thus maintaining the oxygen-content of its deeper layer. He has found that the percentage of oxygen at a depth of 245 meters in Lake Constance is 6.68 c. c. per liter and has shown by experiment (1896, p. 17) that a content of 3.3 c. c. per liter is, if continuously maintained, more than sufficient for the support of sensitive fishes, such as trout. To what extent this oxygen of the deeper layers of water owes its origin to plants of any sort is not known, but there is no reason to believe that any appreciable part of it is due to the larger rooted plants of the shore region. Hoppe-Seyler does not attempt to account for its presence. It is quite possible that the seasonal inversion in which the surface layer is carried to the bottom assists in maintaining the oxygen supply at very great depths. The carbon dioxid present in Lake Constance Hoppe-Seyler found to exist chiefly in the form of carbonates; but little of it (8.14 mg. per liter of water at 147 m. depth) exists free. From these results the conclusion may be drawn with entire definiteness that even at great depths in the lake and very near the bottom only little carbon dioxid is present uncombined, and therefore no hindrance to the respiration of the animals of the lake can occur from the carbon dioxid tension even at such depths.

The observations of Hoppe-Seyler, then, show that the upper layers of the water of the lake to a depth of 2 meters are practically saturated with oxygen, not only where larger aquatic plants are growing, but where there are no such plants. These plants can therefore have no practical effect in increasing the oxygen content of the superficial layer of water. Since his observations show further that in no part of the lake, even at great depths, and in other situations destitute of larger aquatic plants, is there more than a small quantity of uncombined carbon dioxid present, it is clear that the larger plants are not essential for the removal of this gas from the water. It is removed rather as a free gas, by the formation of carbonates. The statement, however, that the larger aquatic plants can not be regarded as essential for the furnishing of oxygen to the animals of a lake or for the removal of carbon dioxid injurious to those animals must be understood as applying only to lakes of considerable size—not to small ponds nor to standing aquaria.

Since the larger plants are scarcely used directly as food by fishes and are of no demonstrated aeration importance in lakes, it remains to determine whether they form one of the links in the chain of nutritive relations that stretches from the water and the soil to the higher fishes; whether, in other words, the plants have, in addition to their mechanical rôle, a nutritive rôle also. If we follow it backward from the fish,

the chain of nutritive relations leads us through the smaller animals chiefly to the microscopic plants, which depend for their food supply upon the carbon dioxid and various other substances in solution in the water. The presence of the other substances is due to various causes; they are brought by tributary streams and by the erosion of the shores; they are washed in from the air by rains, and they come from numerous accidental sources. In solution in the water they are the ultimate sources of food for fish; yet neither fish nor the animals upon which fish feed can secure nourishment from these sources directly. Plants must intervene to organize the mineral salts and carbon dioxid of the water into food.

The aquatic plants may be considered in two groups, one including those which are attached to the soil by roots and the other comprising those which float free or are without organs of attachment. The latter are mostly microscopic, and taken together are designated as the vegetable plankton or phyto-plankton in distinction from the minute free-swimming animals, which as a whole are spoken of as animal plankton or zoö-plankton. In the case of the free plants, food must be obtained from the water which surrounds them, and a deficiency of any one of the substances now known to be essential for plant growth means a reduced quantity of vegetable plankton, and consequently a limited food supply for the fish. The forms of the phyto-plankton require nitrogen, potash, and phosphoric acid just as other plants do, and Brandt (1899) has based upon the work of Apstein the statement that the amount of plankton varies directly with the proportion of nitrates dissolved in the water. The view hitherto usually held has been that the rooted aquatic plants also take their nourishment directly from the water and not at all from the soil; that their roots consequently are organs of attachment only, not organs for drawing nutrition from the soil. If this be true the larger aquatics must, during the growing season, withdraw from the water large quantities of nutritive substances which would otherwise be available for the phyto-plankton, thus lessening the amount of phyto-plankton that the water is capable of producing during this period, and consequently lessening the supply of fish-food dependent on this phyto-plankton. By the subsequent decay of these larger aquatics the food materials withdrawn by them from the water would be returned to it and made available for the phyto-plankton; but while they would thus on the average not lessen, they would, on the other hand, not increase the supply of food for the phyto-plankton.

If, however, the view just expressed be incorrect, and if the larger aquatics draw their supply of mineral food not from the water but from the soil, they draw upon a source which is not available for the phyto-plankton. Their growth, then, does not at any time lessen the supply of phyto-plankton; on the contrary when the larger aquatics

decay, the substances that they have drawn from the soil come into solution in the water and there add to the supply of food available for the phyto-plankton. In thus transferring food materials from the soil to the water these plants would serve a most important function, analogous to the fertilization of land.

According to the usual view, the larger aquatics in their aeration and mechanical rôles tend to increase the supply of fish, while in their nutritive rôle they tend during the growing season to diminish it, though on the average not affecting it. From the alternative view, they tend in all rôles to increase the supply of fish. It thus becomes important to determine the source of nutrition of the larger attached aquatic plants.

### HISTORICAL REVIEW.

Unger (1861) was probably the first to suggest the absorption and excretion of water in submerged aquatics. The existence of amphibious species and those subject to sudden inundation did not escape his notice. It seemed unreasonable to him to suppose that the leaves of amphibious plants, when exposed, should act as organs of transpiration, and, when suddenly submerged, as organs of absorption. He preferred to think that there is an upward current in water plants as well as in land plants, and he endeavored to show that there is a measurable excretion of water by the leaves. He experimented as follows: Two jars filled with water were placed side by side and a U-tube hung on the adjacent edges, so that one shank of the tube descended into each jar. Plants of *Potamogeton crispus* were so arranged that their roots were in one jar, while the stems, passing through the U-tube, were in the other jar. The total leaf surface of the plants was 126 quadricentimeters and they bore 7 adventitious roots several inches long. A preparation similar to the preceding, except that the roots were removed, served for a control. At the end of a week the volume of water in the jars containing the stem portions had in the first-mentioned case increased 1.6 grams, and in the control none whatever. Unger obtained a similar result with *Ranunculus fluitans*.

These experiments were not accepted by Strasburger (1891) and Hochreutiner (1896), although neither of these men makes specific objection. The best reason for not accepting Unger's results is that he fails to show that his method of measurement was sufficiently accurate. An increase of 1.6 grams is a rather small amount, and unless we know that the experimental error must have been less than this the result is to be questioned.

Schenck (1886) states that the roots are primarily organs of attachment, arguing that this must be true since the necessary amount of mineral salts is absorbed directly through the epidermis. This is purely an assumption on the part of Schenck, as there is no experimental evidence to support the view.

Sachs (1887) says: "In algæ, and even in some aquatic phanerogams, the roots are chiefly, or it may be exclusively, organs of attachment."

Frank (1890) observes that while some aquatics swim freely, there are still those whose roots penetrate the substratum and function as do the roots of land plants.

Sauvageau (1891) argues, on page 281, that if one of the uses of the circulation of water in the plant is to supply nutritive substances, this ought to be relatively important in the case of submerged plants, because the water in which they live is oftentimes less rich in dissolved salts than that which circulates in the soil (no authority cited). Continuing, he notes that the roots of certain aquatic plants are well developed. Species of *Potamogeton, Naias*, and *Zostera* have well-developed roots, and the root hairs persist longer than the other cells of the piliferous layer. Species of *Potamogeton* have leaves of two sorts—namely, submerged, without stomata, and exposed, with stomata. On page 282 Sauvageau states that the total surface of the floating leaves is always less than that of the submerged. His hypothesis is that the processes of absorption, conduction, and giving off of water necessitated by the floating leaves are not suddenly initiated at the moment the floating leaves reach the surface, but must have been in operation during the period when the floating leaves were still undeveloped, and likewise in those plants wholly submerged, since their roots serve not only mechanically for attachment, but also for absorption. On page 285 he claims to have demonstrated, by direct measurement of the water passing through the stem of immersed cuttings, that aquatic plants absorb and give off water by a process comparable to that of land plants. It must be noted, however, that in his experiments only fragments of plants were used. In no case did he employ an entire plant with roots. He says that if the plants used had been provided with roots the absorption would have been greater. A careful review of his paper reveals the fact that his conclusion is not warranted. Minden (1899) makes the same objection to Sauvageau's conclusion.

Strasburger (1891) observes that in submerged plants the function of the tracheæ is much diminished; that the salts in the surrounding water may be absorbed by the entire surface of the plant; and that, since there is no transpiration, there is no ascending current. He repeated Unger's experiment, previously described, but failed to get positive results. Instead of using the same plants that Unger used, however, he tried *Ceratophyllum demersum*, and as this plant does not develop roots, his negative result has no significance with regard to Unger's experiment. Moreover, the value of his experiment is doubtful because he speaks of allowing his *Ceratophyllum* plants to take root in flowerpots before beginning the test—an impossible thing, since the plant does not have roots, a fact which he mentions on the preceding page.

Ludwig (1891) gives expression to the current opinion that the roots of aquatic plants serve only for attachment and are without root hairs, and refers to Schenck.

Wieler (1893) states that *Elodea* and *Ceratophyllum* bleed, and since the vascular system of these plants is very rudimentary the movement of water must occur in the intercellular spaces, into which water is forced by adjacent cells, perhaps as in land plants. In consideration of this opinion it is only necessary to note that mere bleeding does not necessarily signify an ascending current, similar to that of land plants.

In the Bonn text-book (Noll, 1902) is the assertion that in general it is true of all submerged aquatics, even phanerogams, that they are able to absorb nutritive solutions through the surface of the whole body, and plants obtaining their food in this way either have no roots or the roots serve merely as mechanical holdfasts.

. Hochreutiner (1896) was the last to investigate the transport of water in submerged plants. His experiment No. 1, with *Ranunculus aquatilis*, illustrates the method employed by him. Two vessels standing adjacent were used, one containing aqueous eosin solution and the other "pure" water. One cutting had its base immersed in the eosin to a depth of 1.5 cm. and its upper portion immersed in "pure" water. A second cutting had 9 cm. of its upper part in eosin and its base in "pure" water. The exposed parts were greased to prevent capillarity and the preparation was kept in a saturated atmosphere. After a day and a half it was found that the eosin could be detected in the main stem of the first plant 9.5 cm. from its base; in a lateral branch 6 cm.; in a leaf 8 cm. The second plant, having 9 cm. of its upper stem in the eosin, showed a coloration in the vascular system through only the apical 3 cm. of the stem. Hochreutiner concludes that in these plants there is an upward current; and although there may be some absorption by the leaves, it is slight compared with that of the roots, these aquatics obtaining their nourishment in the same way that land plants do. He further argues that since there is an upward current there must be also excretion of water by the leaves; and he seems to consider transpiration, or better, exudation, possible in these cases. He endeavored to measure the exudation, but was unable to overcome the practical difficulties. The one objection to Hochreutiner's experiments is that his plants did not have roots[a], and that the eosin entered the exposed vascular system. Although he showed that capillarity would not account for the rate of current, it still remains that conclusions as to the behavior of plants with roots can not be drawn from the behavior of plants without roots.

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[a] Hochreutiner (1896). In a review of this article, in Botanisches Centralblatt, 1898, vol. 68, p. 366, it is stated that the eosin was offered to the roots, but reference to the original shows this is plainly an error. Thus it is probable that A. J. Pieters, Plants of Western Lake Erie (Bulletin U. S. Fish Commission, 1901, p. 73), had access only to the abstract mentioned.

In Vines's Text-book (1896) is the statement that "submerged aquatic plants absorb their food entirely or mainly from the water in which they live." Coulter (1900) agrees with the opinions of Schenck and Strasburger.

The literature thus far reviewed permits one to consider these writers in two groups, one including those whose opinions are derived a priori, assuming that the plants are surrounded by a nutritive solution and that absorption can take place through the epidermis; the other including those who have investigated and who feel warranted in concluding that these aquatics obtain their nourishment by a process comparable to that of terrestrial plants.

Pfeffer (1897) expresses the opinion that a circulation of water in aquatic plants is possible, and he reviews the literature briefly, stating that the experiments of Unger, Wieler, and Hochreutiner are not conclusive, and that the opinions of Strasburger and Sauvageau are not supported by experimental evidence. Also, on page 297, Pfeffer says that no decisive experiments concerning excretion of water in submerged or amphibious plants have yet been made.

The preceding review of literature deals more particularly with the work and opinions of botanists and shows that they are by no means in agreement. On the other hand, however, those who have dealt with the subject from a more general botanical or biological standpoint have given reason for belief that rooted aquatic plants derive nourishment from the soil. Seligo (1890, p. 48) expresses the opinion that the fertility of the bottom should have an effect on the development of shore plants, and points out that in regions where the soil of the adjacent land is fertile the shore region of the lakes is almost everywhere better covered with vegetation than in sterile regions. He then says: "Yet this influence is not so decisive as it appears to be, for a great part of the shore vegetation (algæ) takes its nourishment, not from the bottom, but from the water." Seligo thus, by implication, expresses his belief that larger aquatics draw their nourishment from the soil.

Stockmayer (1894, p. 136) cites a case in which an alga (*Desmonema wrangelii*) appears to depend on a substratum of gneiss.

Pieters (1901, p. 75), in his work on the plants of Lake Erie, showed that there is a probable relation between the abundance of aquatic vegetation and the character of the bottom soil as revealed by mechanical analysis. "As a rule, the soils on which the plants occurred in abundance were composed largely of sand and very fine sand, and contained relatively little silt, fine silt, and clay, while the soils on which few or no plants occurred, although the depth of the water and other physical conditions were favorable, were composed largely of silt, fine silt, and clay, and were poor in fine sand and very fine sand."

Forel (1902, p. 183) says: "In fact, it is classical in botany that aquatic plants are not nourished through their roots, which serve only as organs of attachment; they have no need of humus. Now, however unstable the sand may be, it seems that roots sufficiently deep—nearly all our lacustrine plants have roots—should be able to obtain a sufficient insertion in it. This fact, added to the well-known case of *Elodea canadensis*, which, after having had an abundant vegetation during the first period of its invasion of a new territory, becomes reduced to relatively modest proportions at the end of some years—it seems that it has exhausted the soil—ought not these facts to engage physiological botanists anew in a study of the dogma that the roots of aquatic plants serve only as organs of attachment? It may be possible, however, that they have a certain nutritive function for the plant."

## COMPARATIVE STUDY OF GROWTH UNDER VARYING CONDITIONS OF NUTRITION.

In planning the experiments for this part of the work it was assumed that, other conditions being equal, the one of nutrition determines the volume of vegetation produced. The first endeavor was to determine whether the soil is necessary for optimum growth. For this purpose conditions most nearly approaching the natural ones are desirable, and these are easily obtained in summer by means of floating aquaria, which are described in detail in the succeeding pages.

If the soil is necessary for optimum growth, it may be so chiefly for two reasons, one of these being that it furnishes nourishment, the other that it serves merely as a substratum in which plants may be anchored. In the former case the roots would function as do those of land plants, and in the latter merely as mechanical holdfasts.

If the soil serves merely as a substratum, it would seem that clean washed sand ought to do equally well. For the investigation of this phase of the problem glass aquaria were used, and in these the effects of sand and soil substrata on growth were compared. Further, if the supernatant water tends to extract nutritive salts from the soil, the water above humus soil ought to support a better growth of plants anchored in it than water above clean washed sand. This subject has also received attention, and the methods employed will be described later.

Again, if aquatics do absorb salts through the epidermis, they ought to make an optimum growth in suitable nutritive solutions. The behavior of these plants in culture solutions will also be considered.

## INFLUENCE OF SUBSTRATUM.

### VALLISNERIA SPIRALIS.

This plant occurs usually in water from 15 cm. to 3.5 m. in depth, though Evermann (1902) noted it growing at a depth of 22 feet. It thrives also in shallow running water where a soil substratum is covered by a shallow stratum of gravel and the water remains clear. It prefers a firm soil substratum and never occurs in pure gravel or sand, but its roots will penetrate a thin stratum of sand or gravel to soil beneath, and it may be found rooted in the soil deposits between coarse and loosely lying stones. The roots occur as tufts at the nodes of the creeping rootstock, are fibrous, unbranched, and clothed with root hairs, which are certainly more abundant than is suggested in any literature that has come to my notice. Schwarz (1881–1885) states that one may examine four of five roots of *Vallisneria* before finding root hairs. My observation compels me to differ and to state that this would be exceptional if the plants examined were carefully removed from the soil. On detached and floating specimens exposed to intense light the root hairs soon disappear by death and decay. Schenck (1886a) states that *Vallisneria* and *Elodea* do not develop root hairs, but he is certainly mistaken.

In removing specimens from the soil it is common to find shells pierced by the roots or to find fragments of limestone adhering to them, so it is quite probable that the roots have a corrosive effect upon these insoluble fragments of rock.

The leaves arise from the creeping rootstock, and the older ones have an apical opening similar to that described by Sauvageau (1891) for some other aquatic species. The opening is formed by disintegration of the apical tissue, and results in exposing the vascular system directly to the surrounding medium. In very young leaves this opening could not be found, but it was usually present in leaves 25 cm. or more in length. As a rule, the length of the leaves exceeds the depth of water in which they occur, the upper portion floating horizontally near the surface. This is especially true when the plants are crowded in slowly running water. In the latter case it often happens that the leaves exposed to the intense light turn brown and decay.

*Experiment No. 1.*—This experiment was conducted at the laboratory of the U. S. Fish Commission at Put-in Bay, Ohio, during the period of four weeks from July 18 to August 18. Floating aquaria were constructed as follows: Around the top of two wooden boxes was built a raft large enough to float the boxes, the latter being about 1 m. wide, 1.5 m. long, and 75 cm. deep. In one box was placed a substratum of soil selected from a locality in which *Vallisneria* was abundant. The aquaria were then anchored in the lake and weighted so that they floated, submerged a few centimeters below water surface.

This arrangement furnished the closest approximation to natural conditions. Wooden bars 15 mm. square in cross section and a little less than 1 m. in length

were notched at convenient intervals and upon these the plants were mounted in the following manner: One face of the wooden bar was covered with a ribbon of cheese cloth, fastened with bits of cotton twine which encircled the bar at the notches. A second ribbon of cheese cloth was fastened over the first at one end of the bar. As the plants were placed in the intervals between the notches the outer ribbon of cheese cloth was passed over them and tied to the bar so as to hold them securely. Fifty plants were mounted in this manner, all manipulation being performed under water. Of these 50 individuals 25 were planted in the box containing soil, so that the roots were buried in the mud, the bars being weighted at each end. The remaining 25 plants were placed in the box not containing a substratum. The bars on which the plants were mounted were set horizontally 15 cm. above the bottom of the box.

The plants were taken from the lake by means of a long-handled shovel, with which a portion of soil containing several plants could be raised. By carefully washing away the mud, specimens could be secured without injury to the roots. Young plants of uniform size were selected.

At the end of four weeks in the aquaria the plants were gathered, carefully washed, and air-dried. The total weight of suspended plants was 15 grams and of those



FIG. 1.—*Vallisneria spiralis* after 7 weeks growth rooted in lake soil. Plants in figures 1 and 2 originally the same size.

rooted in soil 20 grams, a difference of $33\frac{1}{3}$ per cent of the former. The plants rooted in soil looked as strong and healthy as those in the lake, and several new individuals had arisen from the rhizomes. The anchored plants did not look so well; only a few new individuals had appeared, and these were stunted in growth. The original plants had grown very little.

*Experiment No. 2.*—This experiment also was conducted at Put-in Bay, Ohio, during the period of seven weeks from July 18 to September 5. It will be noticed that in the preceding experiment, the roots of the suspended plants were exposed to the light prevailing at the depth of 60 cm. That this condition was not a disturbing factor may be inferred from the following experiment:

Two rectangular glass aquaria, each with a capacity of approximately 50 liters, were located on the lake shore. One contained a layer of lake soil 5 cm. deep, the other carefully washed fine gravel from the lake. The same number of plants, uniform in size, was planted in each. The water in the aquaria was siphoned off daily and fresh water from the lake supplied.

At the end of seven weeks a very marked difference could be noticed in the amount of growth of the two sets of plants. Those in gravel were short, bleached, and almost dead. No new shoots had arisen from the rhizomes. The plants in soil were in excellent condition, of good size and color, and 9 new shoots had arisen from the rhizomes. (Compare figures 1 and 2.) It is evident that in both of these experiments the difference in the amount of growth must be attributed to the difference in the environment of the roots.

### RANUNCULUS AQUATILIS TRICHOPHYLLUS.

This species lives wholly submerged in shallow, slowly flowing water. The leaves are finely dissected and incapable of supporting themselves when the plant is taken from the water. The stem branches freely, any branch being able to continue the growth of the plant if the main stem be removed. Roots may arise at any exposed node except, perhaps, the terminal one. If a fragment, a few internodes in length, be detached and left floating roots will arise at the
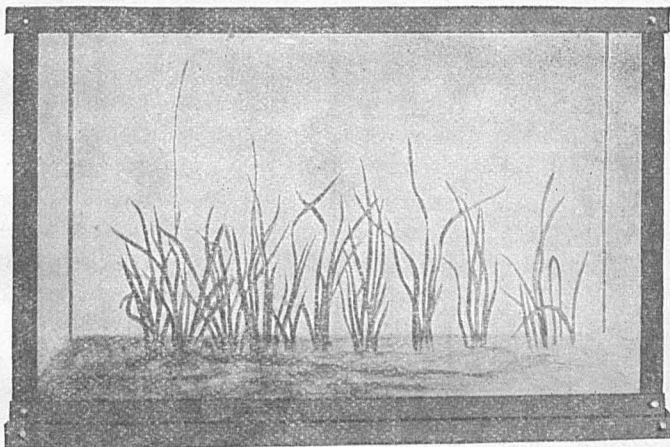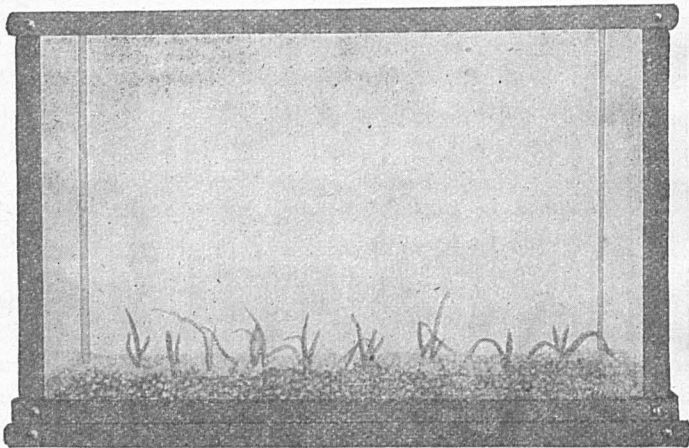


FIG. 2.—*Vallisneria spiralis* after 7 weeks growth rooted in gravel. Plants in figures 1 and 2 originally the same size.

nodes in from six to ten days. These roots grow directly downward, and shortly after entering the soil contract, at least the stem fragment is drawn toward the soil. While the roots are elongating toward the substratum the stem does not elongate, but it quickly resumes growth in length after the roots have entered the soil. More roots then arise from higher nodes, and as those enter the soil the plant is drawn farther down until it is firmly anchored. The roots do not branch before reaching the soil, but do so very shortly after the substratum is penetrated. Numerous lateral roots arise and are formed in succession as the main root advances. A plant with such a root and young lateral roots was carefully removed from the soil and left floating. Neither the main root nor its branches continued to grow, but new roots arose from the upper nodes which again anchored the plant.

The roots are well supplied with hairs; those arising from floating fragments are often almost entirely covered. In one instance a root was found to be clothed with hairs for a distance of 45 cm., which was practically its whole length. The roots are strongly geotropic, and always regain the vertical position if displaced from it.

In each of the following experiments two rectangular glass aquaria of about 50 liters capacity were used. One of these contained a substratum of suitable soil from the bed of a stream and the other contained thoroughly cleaned sand. Considerable pains were taken to remove all the soil particles from the sand, which was accomplished by first washing it as clean as possible, then allowing it to soak for several hours and again washing it, this process being continued until the sand was entirely clean.

A certain number of cuttings from the stock aquaria were planted in the substratum in one end of each aquarium, and in the other end a like number of cuttings were anchored in the supernatant water. To keep these suspended cuttings wholly submerged and in vertical position a small piece of glass tubing was attached by a short cord to the basal node of the cutting. Crystallizing dishes received the roots that developed from the suspended cuttings and prevented their contact with the substratum. By means of a siphon the water in each aquarium was removed on an average of about once a week, fresh water being allowed to enter from the tap above the aquarium as the stale water siphoned out. In this way a complete renewal of water was effected without injury or disturbance to the plants.

*Experiment No. 3.*—In this instance the aquaria were located in the greenhouse at Ann Arbor. The temperature varied from 16° to 22° C., and many of the days were cloudy. The duration of the test was from January 2 to March 5, a period of about sixty days. Terminal portions of plants from the stock aquarium were selected, and 10 such cuttings of uniform length and quality, having neither branches nor roots, were placed in each of the four conditions previously mentioned. After a period of sixty-one days positive differences in the growth of the four groups could be observed.

*Growth measurements of Ranunculus aquatilis trichophyllus at the end of sixty-one days.*
*Original length of each cutting, 15 cm.*

| Condition and specimen number. | Number of internodes on main stem. | Length of main stem. | Number of lateral branches. | Length of lateral branches. | Total length of stem and branches. |
|---|---|---|---|---|---|
| | | *Cm.* | | *Cm.* | *Cm.* |
| 1. Rooted in soil: | | | | | |
| 1............... | 12 | 68 | 5 | 69 | 137 |
| 2............... | 12 | 50 | 8 | 39 | 89 |
| 3............... | 12 | 53 | 4 | 42 | 95 |
| 4............... | 13 | 75 | 6 | 53 | 128 |
| 5............... | 13 | 48 | 7 | 84 | 132 |
| 6............... | 13 | 60 | 3 | 14 | 74 |
| 7............... | 12 | 62 | 5 | 36 | 98 |
| 8............... | 11 | 45 | 0 | 0 | 45 |
| 9............... | 8 | 40 | 3 | 31 | 71 |
| 10............... | 6 | 35 | 3 | 20 | 55 |
| Total ........ | 112 | 536 | 44 | 388 | 924 |
| 2. Rooted in sand: | | | | | |
| 1............... | 13 | 40 | 0 | ............ | ............ |
| 2............... | 16 | 60 | 0 | ............ | ............ |
| 3............... | 14 | 70 | 0 | ............ | ............ |
| 4............... | 13 | 50 | 0 | ............ | ............ |
| 5............... | 14 | 68 | 0 | ............ | ............ |
| 6............... | 12 | 50 | 0 | ............ | ............ |
| 7............... | 14 | 55 | 0 | ............ | ............ |
| 8............... | 12 | 55 | 0 | ............ | ............ |
| 9............... | 14 | 64 | 0 | ............ | ............ |
| 10............... | 14 | 55 | 0 | ............ | ............ |
| Total ........ | 136 | 567 | 0 | ............ | ............ |
| 3. Anchored over soil: | | | | | |
| 1............... | 12 | 42 | 0 | ............ | ............ |
| 2............... | 12 | 31 | 0 | ............ | ............ |
| 3............... | 11 | 32 | 0 | ............ | ............ |
| 4............... | 11 | 31 | 0 | ............ | ............ |
| 5............... | 10 | 26 | 0 | ............ | ............ |
| 6............... | 9 | 28 | 0 | ............ | ............ |
| 7............... | 10 | 28 | 0 | ............ | ............ |
| 8............... | 11 | 35 | 0 | ............ | ............ |
| 9............... | 11 | 30 | 0 | ............ | ............ |
| 10............... | 11 | 30 | 0 | ............ | ............ |
| Total ........ | 108 | 313 | 0 | ............ | ............ |
| 4. Anchored over sand: | | | | | |
| 1............... | 11 | 40 | 0 | ............ | ............ |
| 2............... | 12 | 42 | 0 | ............ | ............ |
| 3............... | 10 | 27 | 0 | ............ | ............ |
| 4............... | 11 | 40 | 0 | ............ | ............ |
| 5............... | 6 | 22 | 0 | ............ | ............ |
| 6............... | 7 | 22 | 0 | ............ | ............ |
| 7............... | 10 | 22 | 0 | ............ | ............ |
| 8............... | 14 | 36 | 0 | ............ | ............ |
| 9............... | 15 | 60 | 0 | ............ | ............ |
| 10............... | 8 | 26 | 0 | ............ | ............ |
| Total ........ | 104 | 337 | 0 | ............ | ............ |

The roots of anchored plants were exposed to light, and this fact must be remembered when comparing these plants with those whose roots entered the substratum. If, however, the roots are only for attachment, then exposure to light should not be a disturbing factor in the amount of growth of the rest of the plant.

Referring to the above tables, the most notable feature is that only one of the cuttings rooted in soil failed to develop lateral branches, while not a single plant of the other three groups developed a lateral branch. Although the total growth of the plants rooted in sand slightly exceeds that of the main stem of those rooted in soil, the large number of lateral branches developed by the latter increases their total

growth to a length greatly in excess of the former.   The two groups
of anchored plants are practically equal in all respects, and it would
seem that the water over sand furnishes as much nourishment as that
over soil.   The plants rooted in sand grew better than those anchored,
but not nearly so well as those rooted in soil.   Lateral roots develop
abundantly in the sand, and thus those plants had a much more exten-
sive root system.

The following percentages, calculated from the tables, afford a con-
venient summary of measurements for comparison.   An allowance of
10 per cent should be made for individual variation unaccounted for.

Comparing with respect to total length:

Plants rooted in soil exceed plants rooted in sand 62.96 per cent of the latter.

Plants rooted in soil exceed plants anchored over soil 195.20 per cent of the latter.

Plants rooted in soil exceed plants anchored over sand 174.18 per cent of the latter.

Plants rooted in sand exceed plants anchored over soil 81.15 per cent of the latter.

Plants rooted in sand exceed plants anchored over sand 68.25 per cent of the latter.

Plants anchored over sand exceed plants anchored over soil 7.66 per cent of the
latter.

<div align="center">POTAMOGETON PERFOLIATUS.</div>

This plant grows wholly submerged at a depth varying from a few
centimeters to a meter.   It is most abundant in protected coves, and is
always found attached to a substratum containing some soil.   Loamy
soil seems to be its first choice, but a fair growth is often attained on
a clayey or sandy bottom.   The plants growing in very shallow
water seldom fruit, while those in the deeper water usually do.   Vege-
tative propagation by creeping root-stocks is conspicuous.   The leaves
are thin, broad, with clasping base, and ribbed.   The plants appear
early in the season and the root-stocks probably remain alive through
the winter.   The growing root-stocks will turn green if left exposed
long enough, and are sensitive to either light or gravitation or to both.
If a cutting of the erect stem be suspended, roots do not arise from the
nodes of the cutting, but instead rhizomes are formed, and from the
nodes of the rhizomes new roots arise.   The roots occur as fibrous
tufts at the nodes of the creeping root-stock and are unbranched.
Root hairs are common, but not so abundant as in *Elodea* or *Ranunculus*.

*Experiment No. 4.*—The location and conditions remain as in the preceding
experiment, the duration being from June 6 to July 25.   In this case the aquaria
stood outdoors instead of in the greenhouse, and to secure a cool substratum and to
prevent the water from becoming too warm they were sunk 10 cm. into the earth.
It was also found necessary to protect the plants from intense light, and this was
done by shading the south side of the aquaria with felt paper, in such manner that
the plants in each received practically the same amount of light.   Water connec-
tions were made with a hydrant, so that fresh water could be supplied, and the stale
water was siphoned out weekly.

Cuttings of terminal portions 15 cm. in length were taken from young and fresh
river plants, and 10 were placed in each of the four conditions used in the preceding
experiment.   These cuttings were without roots or rhizomes, and, in distinction from
the new growth arising from them during the experiment, are designated "original
cuttings."

*Growth measurements of Potamogeton perfoliatus at the end of seven weeks.   Original length of each cutting, 15 cm.*

| Condition and specimen number. | Length original cutting. | Number of nodes on original cutting. | Number of rhizomes. | Total length of rhizomes. | Number of nodes on rhizomes. | Number of secondary shoots. | Total length secondary shoots. | Number of nodes on secondary shoots. |
|---|---|---|---|---|---|---|---|---|
| **1. Rooted in soil:** | *Cm.* | | | *Cm.* | | | *Cm.* | |
| 1 ........ | 20 | 18 | 2 | 70 | 15 | 7 | 170 | 119 |
| 2 ........ | 18 | 17 | 2 | 62 | 12 | 9 | 172 | 128 |
| 3 ........ | 18 | 18 | 1 | 60 | 13 | 8 | 147 | 126 |
| 4 ........ | 16 | 20 | 1 | 54 | 14 | 8 | 222 | 154 |
| 5 ........ | 17 | 18 | 1 | 84 | 17 | 9 | 165 | 126 |
| 6 ........ | 16 | 18 | 1 | 70 | 15 | 7 | 208 | 150 |
| 7 ........ | 17 | 17 | 2 | 116 | 26 | 12 | 304 | 251 |
| 8 ........ | 18 | 19 | 2 | 132 | 25 | 15 | 379 | 261 |
| 9 ........ | 18 | 18 | 1 | 211 | 45 | 21 | 510 | 363 |
| 10 ....... | 16 | 17 | 2 | 190 | 42 | 20 | 487 | 389 |
| Total... | 174 | 175 | 15 | 1,049 | 224 | 116 | 2,764 | 2,017 |
| **2. Rooted in sand:** | | | | | | | | |
| 1 ........ | 17 | 19 | 3 | 35 | 10 | 7 | 31 | 45 |
| 2 ........ | a 28 | 12 | 2 | 46 | 14 | 8 | 22 | 35 |
| 3 ........ | 16 | 20 | 3 | 38 | 9 | 9 | 40 | 52 |
| 4 ........ | 16 | 19 | 2 | 22 | 6 | 6 | 30 | 45 |
| 5 ........ | 15 | 19 | 2 | 40 | 13 | 9 | 30 | 39 |
| 6 ........ | 17 | 18 | 3 | 22 | 5 | 5 | 30 | 27 |
| 7 ........ | 15 | 20 | 2 | 40 | 13 | 9 | 45 | 50 |
| 8 ........ | 18 | 20 | 3 | 39 | 13 | 6 | 43 | 55 |
| 9 ........ | 16 | 20 | 3 | 24 | 13 | 6 | 28 | 39 |
| 10 ........ | 16 | 18 | 2 | 26 | 12 | 5 | 26 | 38 |
| Total... | 174 | 185 | 25 | 332 | 108 | 70 | 325 | 425 |
| **3. Anchored over soil:** | | | | | | | | |
| 1 ........ | 16 | 19 | 2 | 43 | 19 | 7 | 16 | 26 |
| 2 ........ | 17 | 17 | 1 | 30 | 11 | 4 | 8 | 13 |
| 3 ........ | 16 | 19 | 2 | 55 | 20 | 8 | 19 | 32 |
| 4 ........ | 17 | 19 | 2 | 50 | 20 | 9 | 14 | 22 |
| 5 ........ | a 28 | 13 | 2 | 33 | 11 | 6 | 12 | 24 |
| 6 ........ | 15 | 16 | 3 | 48 | 22 | 7 | 9 | 23 |
| 7 ........ | 17 | 20 | 2 | 50 | 18 | 9 | 15 | 30 |
| 8 ........ | 17 | 20 | 2 | 50 | 19 | 10 | 20 | 31 |
| 9 ........ | 15 | 13 | 3 | 30 | 14 | 6 | 12 | 18 |
| 10 ........ | 15 | 15 | 2 | 41 | 17 | 9 | 11 | 19 |
| Total... | 173 | 171 | 21 | 430 | 171 | 75 | 136 | 238 |
| **4. Anchored over sand:** | | | | | | | | |
| 1 ........ | 15 | 20 | 4 | 44 | 20 | 12 | 25 | 81 |
| 2 ........ | 18 | 20 | 3 | 78 | 29 | 16 | 36 | 60 |
| 3 ........ | 16 | 16 | 2 | 50 | 17 | 10 | 16 | 36 |
| 4 ........ | 16 | 22 | 3 | 70 | 24 | 9 | 23 | 53 |
| 5 ........ | 16 | 16 | 3 | 50 | 20 | 9 | 17 | 45 |
| 6 ........ | 18 | 20 | 2 | 56 | 19 | 10 | 27 | 56 |
| 7 ........ | 16 | 14 | 2 | 50 | 18 | 7 | 15 | 36 |
| 8 ........ | 16 | 19 | 3 | 51 | 25 | 10 | 22 | 48 |
| 9 ........ | 18 | 20 | 3 | 81 | 29 | 12 | 34 | 62 |
| 10 ........ | 16 | 20 | 3 | 63 | 20 | 10 | 26 | 39 |
| Total... | 165 | 187 | 28 | 593 | 221 | 105 | 241 | 466 |

*a* Fruited.

*Summary of results in four conditions.*

| Items. | Rooted in soil. | Rooted in sand. | Anchored over soil. | Anchored over sand. |
|---|---|---|---|---|
| | *Cm.* | *Cm.* | *Cm.* | *Cm.* |
| Average length of rhizome ..................................... | 69.93 | 13.28 | 20.5 | 21.2 |
| Average length of rhizome-internode .........................• .............. | 4.7 | 3.07 | 2.45 | 2.7 |
| Average length of secondary shoots ............................. | 23.82 | 4.64 | 1.8 | 2.8 |
| Average length of internode of secondary shoots.............. | 1 3 | .77 | .57 | .5 |

From these tables it will be noted:

1. The original cuttings in each of the four conditions practically ceased to grow in length early in the experiment, adding on the average less than 3 cm. to the original 15 cm.

2. The new growth consisted of rhizomes and secondary shoots arising from them.

3. The plants rooted in soil produced on the average fewer rhizomes than those in any of the other three conditions.

4. The average length of the rhizomes arising from the plants rooted in soil greatly exceeded that of the rhizomes arising from the plants in each of the other three conditions.

5. The average length of the secondary shoots from the plants rooted in soil greatly exceeded that of the secondary shoots from the plants in the other three conditions.

6. The plants anchored over sand averaged about equally in all respects with those anchored over soil.

7. The plants rooted in sand exceeded in all respects, except the length of rhizome, the two groups of anchored plants.

In this species the habit of the plant persists whether the cuttings be in sand, in soil, or anchored, and the differences arising from the differences of environment are quantitative rather than qualitative. All of the plants produced rhizomes and secondary shoots. In *Ranunculus aquatilis trichophyllus*, however, it will be remembered that the natural habit of the plant persisted only in the individuals rooted in soil, lateral branches failing to develop in the other groups.

## MYRIOPHYLLUM SPICATUM.

Quiet water 1 to 2 meters deep and a good loamy soil are the favorite habitat of this species. Isolated specimens occur in shallow water and sandy soil where they have been washed as drifting fragments, but the plants do not establish themselves under such conditions. Long branching roots are developed, but root hairs have never been found. Roots may arise at almost any node, and numerous stem branches arise to give the plant a bushy form. The leaves are finely dissected, the stem strong and flexible, so that the plant seems adapted to rougher water than that in which it usually occurs. I have never found it occupying any considerable area or so abundant as to suggest the exclusion of other species by it. As roots develop abundantly, but do not have root hairs, it was considered desirable to determine whether or not the plant is dependent upon its attachment to the soil for optimum growth.

*Experiment No. 5.*—The location and conditions remain as in experiments 3 and 4. The duration in this case is one month, from July 10 to August 10. Terminal cuttings 15 cm. in length and without roots were selected from thrifty river plants.

On August 10 the general appearance of the plants was as follows: The two groups of anchored plants were about alike in all respects and had numerous roots arising from

the 5 or 6 lowest nodes. These roots had no branches. The plants rooted in sand had numerous roots which were longer than those of the anchored plants, profusely branched and white. The plants rooted in soil were about equal to those rooted in sand in root development, but the roots were of a dark purple color, which is common, though not universal, in wild specimens. None of the roots arose from nodes above earthy substratum. The internodes in all cases were of about equal length. The only difference seemed to be merely that there was more growth in the plants rooted in soil.

*Growth measurements of Myriophyllum spicatum at the end of 31 days. Original length of each cutting, 15 cm.*

| Specimen number. | Rooted in soil. | Rooted in sand. | Anchored over soil. | Anchored over sand. |
|---|---|---|---|---|
| | *Cm.* | *Cm.* | *Cm.* | *Cm.* |
| 1 | 55 | 38 | 38 | 55 |
| 2 | 79 | 41 | 31 | 47 |
| 3 | 84 | 38 | 35 | 38 |
| 4 | 44 | 57 | 36 | 45 |
| 5 | 54 | 59 | 36 | 66 |
| 6 | 50 | 60 | 46 | 42 |
| 7 | 75 | 35 | 28 | 20 |
| 8 | 77 | 39 | 39 | 33 |
| 9 | 84 | 35 | 27 | 22 |
| 10 | 76 | 61 | 34 | 30 |
| Total | 678 | 463 | 350 | 398 |

The measurements in the accompanying tables show:

(1) A positive difference in favor of plants rooted in soil.

(2) The two groups of anchored plants are practically alike.

(3) The plants rooted in sand exceed those anchored, but do not approach in growth those rooted in soil.

### ELODEA CANADENSIS.

Either still or running water is suitable for *Elodea.* It grows attached to the substratum by adventitious roots arising at the nodes. I have never found lateral branches on the roots, although I have made several attempts to do so. The plant thrives in shallow or deep water and seems to be adapted to light of varying intensity. When growing in water a meter or two in depth the internodes are noticeably longer, the stem thicker and less branched. Roots arise quickly from the nodes of a drifting fragment. At Put-in Bay a large thrifty plant was found afloat, which bore a single root 90 cm. in length. *Elodea* likes a good loamy soil. It does occur in clay, and may frequently be noticed growing clustered in what appears to be a sand substratum, but I have always found some humus soil present in such cases.

*Experiment No. 6.*—The location and conditions are continued here as in preceding experiments, the duration being one month, July 10 to August 10. Terminal cuttings 10 cm. long were selected from fresh river specimens. These cuttings were alike in all respects, and were without roots or branches.

On August 10 little difference, if any, could be noticed in the plants rooted in sand, anchored over soil or anchored over sand. The diameter of the stem and the

length of internode were about the same for all, and all of the plants were of fairly good green color. Those rooted in soil were, in comparison, of a more delicate green and in first-class condition. The stem was less in diameter and the internodes markedly longer. The accompanying table shows the total length at the end of one month. As only a few branches and rhizomes developed, these are included in the total for each plant.

*Growth measurements of Elodea canadensis at the end of 31 days. Original length of each cutting, 10 cm.*

| Specimen number. | Rooted in soil. | Rooted in sand. | An-chored over soil. | An-chored over sand. |
|---|---|---|---|---|
|  | *Cm.* | *Cm.* | *Cm.* | *Cm.* |
| 1 | 117 | 36 | 26 | 35 |
| 2 | 110 | 28 | 39 | 27 |
| 3 | 118 | 29 | 18 | 45 |
| 4 | 220 | 27 | 23 | 40 |
| 5 | 184 | 34 | 24 | 41 |
| 6 | 165 | 30 | 32 | 20 |
| 7 | 171 | 42 | 29 | 38 |
| 8 | 160 | 45 | 40 | 33 |
| 9 | 143 | 30 | 31 | 24 |
| 10 | Died. | 49 | 22 | Died. |
| Total | 1,388 | 350 | 284 | 303 |
| Average | 154.2 | 35 | 28.4 | 33.6 |

The table shows:

(1) An approximate equality of the anchored plants with one another.

(2) A great difference in favor of the plants rooted in soil.

(3) The plants rooted in sand exceed the anchored plants, but hardly enough to establish a positive difference.

### CHARA.

*Experiment No. 7.*—August 20 to September 15. Location and conditions as in preceding. This plant being an alga and much simpler than any of the preceding species in organization, and having rhizoids instead of roots, it seemed probable that it would be found to be independent of a soil substratum for optimum growth. Terminal cuttings 15 cm. long were selected as in preceding cases and the same experiment tried.

*Growth measurements of Chara at the end of 26 days. Original length of each cutting 15 cm.*

| Specimen number. | Rooted in soil. | Rooted in sand. | An-chored over soil. | An-chored over sand. |
|---|---|---|---|---|
|  | *Cm.* | *Cm.* | *Cm.* | *Cm.* |
| 1 | 32 | 39 | 20 | 38 |
| 2 | 42 | 27 | 24 | 25 |
| 3 | 56 | 20 | 24 | 21 |
| 4 | 36 | 20 | 39 | 32 |
| 5 | 32 | 22 | 23 | 23 |
| 6 | 40 | 26 | 24 | 16 |
| 7 | 46 | 52 | 28 | 21 |
| 8 | 43 | 45 | 22 | 50 |
| 9 | 35 | 30 | 23 | 28 |
| 10 | 42 | 33 | 34 | 34 |
| Total | 454 | 314 | 261 | 288 |

It will be noticed that the ratios of the respective amounts of growth are the same as for the other plants tried. Those rooted in soil grew most, those rooted in sand next, those anchored over sand about equally with those suspended over soil, and both less than those rooted in sand or in soil.

### CERATOPHYLLUM DEMERSUM.

Roots are not present in this plant. The rudiment of a root exists in the embryo, but does not develop when the seed germinates. Growing thus without roots, the plant is easily carried by waves and currents to various habitats, but it occurs most abundantly where least disturbed, usually in protected coves where the water is a meter or two in depth. The finely dissected leaves are borne in whorls, and the segments are rather rigid, so that a plant dragging on the substratum is likely to become anchored. In a sheltered cove where it grows abundantly one may carefully pull up long specimens and usually find that a portion of the stem has been buried and a more or less vertical position secured for the plant. Sometimes the central portion of the axis is buried in the soil so that the two ends of the plant are free. The buried portion is simply bleached; no indications of adventitious organs can be noted.

*Experiment No. 8.*—Aquarium tests were made with this plant as in the cases preceding. Sufficiently uniform figures for the individuals of a given group were not obtained. Some of the plants in soil grew more than some of those in sand, and conversely. Likewise, the two groups of anchored plants were not comparable with each other, nor with those in sand or soil. In view of such results and the fact that no specialized organs of attachment are produced, it is reasonable to consider that this plant is not directly dependent upon the soil for its growth.

### POTAMOGETON OBTUSIFOLIUS.

*Experiment No. 9.*—It was intended to grow this plant as material for chemical analysis, cuttings being selected from fresh river specimens and placed in floating aquaria as described for *Vallisneria* (experiment No. 1, p. 494). These aquaria were anchored in slowly flowing water in the Huron River, Ann Arbor, on August 14 and remained until September 12. By the latter date the plants were so incrusted as to be disqualified for analysis and only the general result may be recorded.

The difference in favor of the plants rooted in soil was very positive. They had elongated and grown considerably—in fact, behaved as though growing naturally. The suspended plants had failed to grow and showed signs of succumbing to adverse conditions. They had produced numerous unbranched roots, but these decayed after reaching a length of 25 or 30 cm. No rhizomes were produced in either case, the new growth being merely a continuation of branches present when the cutting was made. It may safely be said that this species also is dependent upon the soil for optimum growth.

### VALLISNERIA AND CHARA.

*Experiment No. 10.*—This experiment was conducted at Put-in Bay, Ohio, during the period from August 7 to September 14. Having observed that wherever *Vallisneria* grows best a certain type of soil is likely to be found, it was considered desirable to select the three most distinct types of soil occurring in the vicinity and to tets

them as to the amount of vegetation each can support. To secure natural conditions a platform was built in the lake near the laboratory and on this platform were placed three glass aquaria. The tops of the aquaria were about 15 cm. below the lake level. Each aquarium contained one type of soil as a substratum of about 10 cm. depth. In each aquarium 10 plants of *Vallisneria* and 10 of *Chara* were planted. This material was carefully selected, the individuals being of uniform size, placed in water of favorable depth, and exposed to natural light conditions.

The following table gives the mechanical analysis of the three types of soil as determined by the Bureau of Soils, U. S. Department of Agriculture. The results are expressd in percentages:

*Analyses of soils tested for growth of Vallisneria and Chara.*

| Items. | No. 1. | No. 2. | No. 3. |
|---|---|---|---|
| | *Per ct.* | *Per ct.* | *Per ct.* |
| Soluble salts as determined by mechanical analysis | 0.42 | 0.69 | 0.74 |
| Organic matter | 6.50 | 8.02 | 4.22 |
| Gravel, 2 to 1 mm | 8.78 | .84 | 1.54 |
| Coarse sand, 1 to 0.5 mm | 3.40 | .62 | 2.12 |
| Medium sand, 0.5 to 0.25 mm | 2.84 | .90 | 1.96 |
| Fine sand, 0.25 to 0.1 mm | 12.20 | 19.40 | 12.44 |
| Very fine sand, 0.1 to 0.05 mm | 3.02 | 13.30 | 9.90 |
| Silt, 0.05 to 0.005 mm | 47.94 | 47.56 | 36.10 |
| Clay, 0.005 to 0.001 mm | 14.26 | 8.05 | 31.04 |

The following notes, taken by Prof. F. C. Newcombe, furnish a general characterization of the three soils as determined by observation:

No. 1. Brownish gray throughout, cohesive, very fine texture, little if any grit to the feeling, abundant plant remains in fine fibres, no gas in hydrochloric acid.

No. 2. Blackish gray, gritty, rather coarse, sandy, cohesive, fibrous with plant remains, molluscan shells sparse, yielding much gas in hydrochloric acid.

No. 3. Bluish clay, blotched with buff, hard and coherent, almost no grit, few plant remains, little gas in hydrochloric acid. After the action of acid a granular sediment remains composed apparently of quartz grains.

The experiment shows that soil No. 1 supports the most growth, soil No. 2 next, and soil No. 3 the least growth. The same relation holds for *Chara* as for *Vallisneria*. The relative size of representative plants from each of the three aquaria is shown in figures 3, 4, and 5. The plants were pressed and mounted, the photographs being taken from the herbarium sheet.



FIG. 3.—*Vallisneria spiralis* after 5½ weeks' growth in loamy soil (No. 1).

*Chara* being difficult to subject to linear measurement, the air-dry weight of the 10 plants in each case was taken, and this gives a fair index of the relative amount of growth in each soil. In No. 1 it was 2.175 grams; in No. 2, 1.345 grams; in No. 3, 0.650 grams.
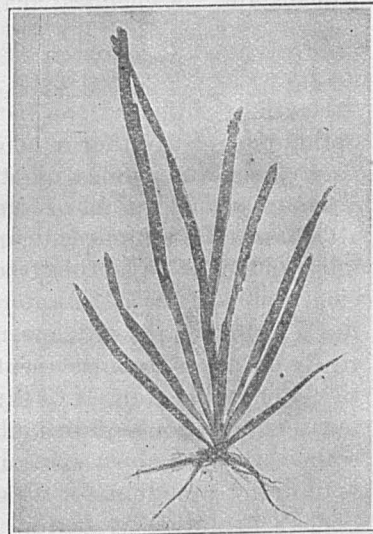
The result for *Vallisneria* is just what was expected, but in the case of *Chara* it was thought that since it is of more frequent occurrence in the sandy soil, perhaps it would make a better growth in No. 2 than in No. 1. It is quite possible that this plant is unable to hold possession of the soil of its choice because of the interference from other species. It does occur infrequently along with *Vallisneria* in the loamy soil, making excellent growth there, and since experiment shows this soil to be more favorable than that in which it frequently occurs, we might suppose that it is crowded out from places otherwise suitable for it.
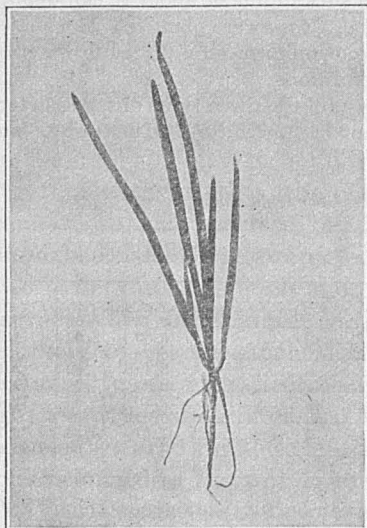


FIG. 4.—*Vallisneria spiralis* after 5½ weeks' growth in sandy soil (No. 2).

Looking to the mechanical analysis as shown above for explanation of these results, it is difficult to find differences indicating those properties which are determining factors in the amount of growth a given soil will sustain. A chemical analysis also is probably necessary.

### GROWTH IN NUTRIENT SOLUTIONS.

Having established the fact that certain aquatics do not make an optimum growth either in lake water or ordinary river water unless rooted in the soil, although a substratum of sand and artificial attachment be supplied, it remains to determine whether this fact may be due to insufficient nourishment in the water. Again, from the a priori point of view, if these plants really do absorb nourishment over their entire surface, they ought to thrive in artificial nutrient solutions of suitable strength and composition. Knop's solution was tried, but is too good a medium for the growth of algæ. Sachs's[a] solution is better, and, although osmotically stronger than tap water, is still safe within the limit of suitable strength.



FIG. 5.—*Vallisneria spiralis* after 5½ weeks' growth in clay soil (No. 3).

In the two succeeding experiments two species of plants were grown in each of five conditions, namely:

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[a] Sachs's solution is, $KNO_3$, 1 gram; $CaSO_4$, 0.5 grams; $MgSO_4$, 0.5 grams; NaCl, 0.5 grams; $Ca_3(PO_4)_2$, 0.5 grams; dissolved in water to 1 liter.

(1) Soil and tap water, (2) sand and tap water, (3) tap water without substratum, (4) Sachs's solution without substratum, and (5) Sachs's solution with sand substratum.

#### ELODEA CANADENSIS.

*Experiment No. 11.*—This experiment was conducted at Ann Arbor during the period from July 10 to August 10. Ten cuttings 10 cm. long, anchored with bits of glass tubing, were suspended in each of five cylindrical battery jars of about 3.25 liters capacity, containing 3 liters of solution with substrata, as already mentioned and as designated in the table. The jars were covered with netting to keep out insects and foreign matter, and, to maintain a sufficiently cool temperature, were sunk in the earth out of doors to within about 6 cm. of the top of the jar. The salts in the Sachs's solution were present in the same proportion as in the formula, and the solution was renewed weekly to prevent the growth of algæ. The tap water was of course likewise renewed.

*Measurement of growth of Elodea canadensis at the end of one month. Original length of each cutting, 10 cm.*

| Specimen number. | Soil and tap water. | Sand and tap water. | Tap water only. | Sachs's solution only. | Sachs's solution and sand. |
|---|---|---|---|---|---|
| | *Cm.* | *Cm.* | *Cm.* | *Cm.* | *Cm.* |
| 1............ | 36 | 19 | 27 | 16 | 30 |
| 2............ | 35 | 28 | 28 | 17 | 25 |
| 3............ | 58 | 16 | 25 | 16 | 29 |
| 4............ | 35 | 22 | 22 | 18 | 31 |
| 5............ | 30 | 33 | 21 | 23 | 30 |
| 6............ | 34 | 27 | 18 | 14 | 29 |
| 7............ | 29 | 24 | 19 | Died. | 33 |
| 8............ | 40 | 26 | 21 | Died. | 23 |
| 9............ | 34 | 25 | Died. | Died. | 25 |
| 10............ | 54 | Died. | Died. | Died. | Died. |
| Total .... | 385 | 220 | 181 | 104 | 255 |
| Average. | 38.5 | 24.4 | 22.6 | 17.3 | 28.3 |

It will be noticed from this table that four of the plants in Sachs's solution died before the experiment was concluded, and it is certainly evident that the normal growth of the plants was greatly interfered with. Of the five conditions tested, that of soil and tap water is certainly the best, while that of Sachs's solution without substratum is the least favorable. The remaining three conditions can not be said to show positive differences.

It is a noteworthy fact that not a single root developed on the cuttings anchored in Sachs's solution. Only a few developed in Sachs's solution with sand substratum, while in all the tap-water jars the development of roots was abundant.

POTAMOGETON PERFOLIATUS.

*Experiment No. 12.*—The location and conditions remain as in the preceding experiment. The duration of the experiment was from August 17 to September 15. Terminal portions, 10 cm. in length, were selected from fresh river plants, and 6 cuttings used in each case. The following table shows the measurements at the end of 27 days:

*Measurement of growth of Potamogeton perfoliatus at the end of 27 days. Original length of each cutting, 10 cm.*

| Specimen number. | Soil and tap water. | Sand and tap water. | Tap water only. | Sachs's solution only. | Sachs's solution and sand. |
|---|---|---|---|---|---|
|  | *Cm.* | *Cm.* | *Cm.* | *Cm.* | *Cm.* |
| 1............... | 115 | 34 | 50 | 20 | 28 |
| 2............... | 83 | 41 | 49 | 24 | 30 |
| 3............... | 130 | 63 | 36 | 24 | 33 |
| 4............... | 105 | 55 | 57 | 32 | 32 |
| 5............... | 82 | 35 | 39 | 25 | 26 |
| 6............... | 80 | 55 | 40 | 24 | Died. |
| Total ... | 595 | 283 | 271 | 149 | 149 |
| Average | 99.16 | 47.33 | 45.16 | 24.83 | 29.8 |

The measurements show—

(1) That for this plant, also, soil and tap water furnishes the most favorable of the five environments tested. In this case. the plants behaved as under corresponding conditions in the aquarium experiments and as they do in nature. The original cutting grew very little, the increase of growth coming from new rhizomes and secondary shoots from them.

(2) That Sachs's solution furnishes the least favorable of the five environments tested.

(3) That tap water either with or without sand is inferior to soil and tap water, but superior to Sachs's solution.

(4) That in this experiment the differences are decisive, and it is possible that another test would show *Elodea* to behave more nearly like *Potamogeton*.

It was also noted that root development in this species is greatly inhibited, although not completely suppressed, as in the case of *Elodea*, by Sachs's solution.

RANUNCULUS AQUATILIS TRICHOPHYLLUS.

*Experiment No. 13.*—This experiment was conducted in the greenhouse at Ann Arbor during the period from November 22 to December 26. Three conditions were established. As the plants seem to do better in Sachs's solution when the sodium chloride is absent, this salt was omitted in this experiment. The nutrient solution was identical in each condition, but one jar contained a soil substratum, another sand, and the third was without substratum. The jars stood in the greenhouse and the solutions were renewed weekly. Six cuttings, 10 cm. in length, were selected from the stock aquarium and planted in each jar, those in the jar without

substratum being suspended and anchored with bits of glass tubing attached. The accompanying table shows the increase in length of the plants after a period of 34 days:

*Growth measurement of Ranunculus aquatilis trichophyllus at the end of 34 days. Original length of each cutting, 10 cm.*

| Specimen number. | Anchored without substratum. | Rooted in sand. | Rooted in soil. |
|---|---|---|---|
| | *Cm.* | *Cm.* | *Cm.* |
| 1....................... | 20 | 20 | 28 |
| 2....................... | 19 | 18 | 27 |
| 3....................... | 19 | 18 | 28 |
| 4....................... | 20 | 18 | 26 |
| 5....................... | 18 | 19 | 23 |
| Total ............. | 115 | 118 | 156 |
| Average ......... | 19.16 | 18.83 | 26 |

These figures show that a soil substratum favors the growth even in a nutrient medium which contains all the necessary nourishment.

Sachs's solution inhibits the root development here also, but not nearly so much as with *Elodea* and *Potamogeton*. As all three of these plants develop roots abundantly in tap water without a substratum, we may suppose that Sachs's solution contains ingredients which are unsuitable, at least in the given proportion. While it is true that in all my experiments a good plant growth is accompanied by a well-developed root system, it will be remembered that in the aquarium experiments with *Ranunculus* the plants rooted in sand had a good root system, but not a corresponding growth of stem. Although Sachs's solution may be regarded as unfavorable for root development, we can not attribute the retarded growth of the plant to an injurious effect from it unless we assume that the injury is neutralized by the soil, for we get a much better growth in Sachs's solution over a soil substratum.

## SIGNIFICANCE AND DISTRIBUTION OF ROOT HAIRS.

Since a root hair is merely a peripheral root cell protruded, the interpretation generally accepted for this structure is that it serves to increase the absorbing surface of the root. If this is correct, and the roots of aquatic plants are only for mechanical attachment, a root hair would seem to be an unnecessary structure in such species.

Many authors have made much of the fact that submerged aquatics show a very rudimentary vascular system and that their anatomy indicates that absorption is not a specialized function of the plant. Perhaps this is somewhat justifiable, but have we not in the presence of root hairs reason for a different opinion? These are such simple structures that they would not be likely to be developed very long after becoming unnecessary; i. e., after the plants bearing them had passed from terrestial to aquatic habits.

Root hairs have been found on the following plants:[a]

Elodea canadensis Michaux.    *Philotria canadensis* (Michaux) Britton.
Naias flexilis Rostkovius & Schmidt.
Naias flexilis robusta† Morong.
Potamogeton pectinatus Linnæus.    *Potamogeton filiformis* Persoon.
Potamogeton pauciflorus Pursh.    *Potamogeton foliosus* Rafinesque.
Potamogeton gramineus Linnæus.    *Potamogeton heterophyllus* Schreber.
Potamogeton lucens Linnæus.    *Potamogeton lonchites* Tuckerman.    *Potamogeton zizii* Roth.
Potamogeton natans Linnæus.
Potamogeton perfoliatus Linnæus.
Potamogeton prælongus Wulfen.
Potamogeton zosteræfolius Schumacher.
Ranunculus aquatilis trichophyllus Gray.    *Batrachium trichophyllum* (Chaix) Bossch.
Vallisneria spiralis Linnæus.

The following do not develop root hairs, but are well provided with roots:

Bidens beckii Torrey.
Heteranthera graminea Vahl.    *Heteranthera dubia* (Jacquin) MacMillan.
Myriophyllum sparsiflorum Wright.    *Myriophyllum spicatum* Linnæus.

## BEHAVIOR OF ROOTS AS ORGANS OF ABSORPTION.

### ABSORPTION OF LITHIUM NITRATE.

While the experiments already described render the absorption of mineral salts by the roots highly probable, it is of course desirable to secure more direct evidence.    For this purpose two methods were employed: First, a 1 per cent solution of lithium nitrate in tap water was offered to the roots, and after a time the upper parts of the plant were tested for lithium with the flame and spectroscope.    The second method was merely a direct measurement of the water absorbed.

#### RANUNCULUS AQUATILIS TRICHOPHYLLUS.

*Experiment No. 14.*—This was performed in the greenhouse on February 7.  A cutting was taken from the stock aquarium and allowed to grow roots which were straight, unbranched, intact, and clothed with hairs.  The plant ready for the test may be described as follows: Distance from the node at the base of the cutting to the terminal node, 20 cm.; from the node at the base of the cutting descended one root 20 cm. in length; from the first node above the basal node of the cutting descended one root 10 cm. in length.

As the test must be made with the plant submerged, it is very necessary that none of the lithium nitrate solution escape from the containing bottle into the surrounding water.    To separate root and stem an adequate stopper was made by saturating cotton in melted vaseline.    Such a stopper can be wrapped around the stem until it snugly fits the bottle.    The vaseline makes it water-tight and prevents capillarity along the stem, yet does not injure the plant.

[a] The nomenclature of this list is that of the Index Kewensis, a dagger indicating a more recently established species, and the italicized names the synonyms.

The base of the cutting, including the adjacent portion of the root, being wrapped in the stopper, the plant was located with the longer root inside a narrow-mouth bottle partly filled with lithium nitrate solution. The preparation was then submerged in an aquarium, this arrangement leaving the shorter root outside the bottle and serving as a check on diffusion from the bottle. The distance from the base of the cutting to the level of the solution in the bottle being 4 cm., any lithium escaping from the bottle except through the tissues of the plant would have to do so by capillarity along this root. The preparation was left standing twenty-four hours. The temperature was 17° C. and the weather cloudy.

Upon examination lithium was found in all parts of the stem and leaves, except the terminal node and leaf. No lithium could be detected in the root outside the bottle, not even within 2 millimeters of its union with the stem.

*Experiment No. 15.*—This experiment was also performed in the greenhouse, the date being March 2. In this case conditions were the same as in the preceding experiment, except that the cutting was allowed to root in a sand substratum and develop numerous lateral roots. This furnished a normal root system, and thus better material for securing an indication of the probable rate of current in the plant. After the plant was well rooted the sand was carefully washed away with as little injury to the roots as possible. The cutting was then left suspended for three weeks to allow any injuries to the roots to heal. The stem of the cutting from base to tip measured 40 cm. Two roots, well branched, descended from the basal node. Both of these roots were placed in the bottle. Other roots arising from higher nodes were left outside the bottle. The distance from the base of the stem to the level of the lithium nitrate solution was 4 cm. The duration of the test was 11.30 a. m. to 4.30 p. m., the temperature 20° C., the sky clear.

Examination revealed the fact that the lithium had traveled upward a distance of 17 cm. from the level of the solution, or 13 cm. in the stem and 4 cm. in the roots. Not a trace of lithium could be found in the roots outside the bottle. One of these roots joined the stem two internodes below the highest point in the stem reached by the lithium. As the lithium had gone upward only 13 cm. out of a possible 40 cm., it is reasonable to assume that these figures approximate the rate of current in the plant.

Mere diffusion will not account for these results, for if the process were simply that, why should not the roots outside the bottles have at least a trace of lithium in the portion close to the stem axis in which the salt was present in abundance? Mere diffusion of salts takes place more rapidly downward than upward.

## MEASUREMENT OF ROOT ABSORPTION.

### RANUNCULUS AQUATILIS TRICHOPHYLLUS.

*Experiment No. 16.*—This was performed in the greenhouse in March. By this method the amount of water absorbed by the root is measured directly. The root is inclosed in a bottle (figure 6) provided with an indicating tube in which the water level falls as absorption by the root proceeds. A very simple preparation proved adequate for this purpose. A rubber stopper was pierced with a steel wire and the projecting end of the wire heated until the rubber melted to form a perforation of the desired diameter. The stopper was then divided under water with a sharp razor, a very smooth cut being absolutely necessary. The accompanying figure shows the plan of the apparatus. The indicating tube rises above the level of the water in the aquarium and descends to the level of the stopper's base, so that air bubbles may have an easy exit.

The bottle having been immersed in the aquarium, the root is inclosed by the two halves of the stopper and the preparation set up as figured. Air must be excluded from the bottle and indicating tube. The water level in the tube and that in the aquarium must coincide when the experiment begins. If the preparation is successful a change of temperature in the aquarium water will cause a corresponding change in the level of water in the tube. After this test is made and a uniform temperature established, the experiment may begin. When the experiment is concluded the water level in the aquarium must be the original level and the original temperature must be secured. If under these conditions the level of the water in the tube is below the level of the water in the aquarium, the root must have absorbed a volume of water equal to the volume of the tube contents for the distance between the last level and the level of the water in the aquarium.

The stem axis of the plant used was 20 cm. in length and had 5 nodes with leaves. The cutting bore one straight, unbranched, intact root 14 cm. in length and clothed
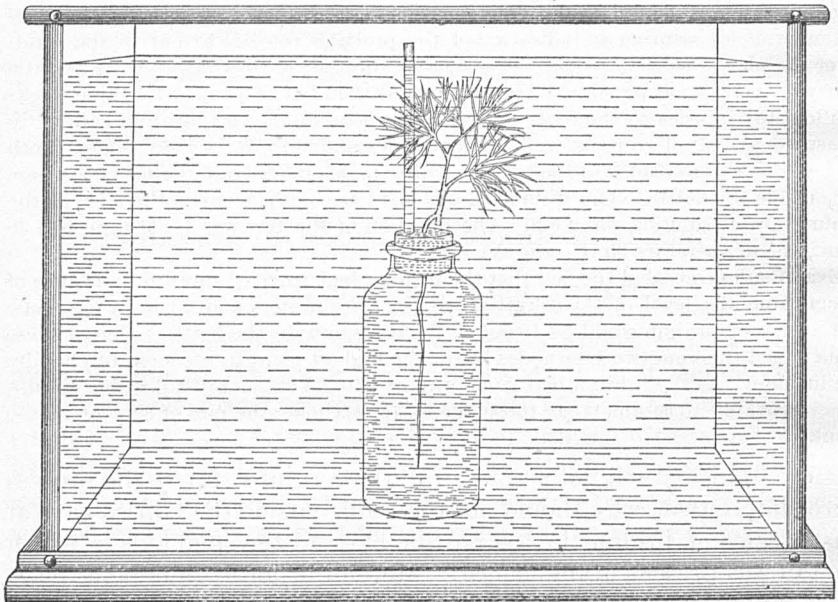


Fig. 6.—Apparatus for measuring root absorption.

with hairs. The water in the tube dropped 15 cm., equal to a volume of 5 c. c., and hence the root absorbed from the bottle this amount of water in twenty-four hours. This test was repeated the following day, the tube receiving the 5 c. c. necessary to make the levels coincide again, and the same result was obtained. It thus appears that a plant of this description at a temperature of 20.5° C. absorbs about 5 c. c. in twenty-four hours.

That the roots do absorb water is therefore considered to be established by two direct methods. One step further would be to measure the water excreted by the stem and leaves. Numerous efforts were made to accomplish this, but no satisfactory apparatus could be devised that would secure reliable results.

## CHEMICAL ANALYSIS.

Chemical analysis has been employed for the purpose of securing, if possible, some clew to the reason why certain plants can not make normal growth unless rooted in soil. *Vallisneria* was selected as the material for analysis, and two sets of plants were grown in floating aquaria (the same as described for experiment No. 1), stationed in the lake at Put-in Bay. One aquarium contained anchored plants only; the other contained plants rooted in a soil substratum, and the plants were under these conditions for five weeks during July and August. At the close of this period each set of plants was gathered and thoroughly washed in running lake water. All unhealthy individuals were discarded. The fresh volume of each set was obtained by immersion in water, and came so near being the ratio of 2 to 1 that a few of the suspended plants were left out to secure the ratio. The fresh volume of plants rooted in soil was 1,380 c. c. and of those suspended 690 c. c. This material was then air dried and sent to the U. S. Department of Agriculture for analysis, the results of which are recorded in the following table:

*Analysis of Vallisneria spiralis.*

| • | Rooted in soil. | Anchored. |
|---|---|---|
| Total weight of material air-dried ................................grams.. | 52.70 | 87.20 |
| Moisture in material air-dried .............................per cent.. | 9.95 | 11.45 |
| Total dry weight, moisture deducted.........................grams.. | 47.46 | 32.94 |
| Ether extract in air-dried material ........................per cent.. | 2.51 | 1.63 |
| Crude fiber........................................................do.... | 16.97 | 15.73 |
| Ash................................................................do.... | 20.34 | 17.45 |
| Starch.............................................................do.... | 2.89 | 6.75 |
| Protein............................................................do.... | 16.81 | 13.44 |
| Pentosans and ligno-cellulose, by difference...................do.... | 31.03 | 33.55 |

Keeping in mind that the fresh volume of the anchored plants was just one-half that of the plants rooted in soil, it will be noticed that this ratio is not sustained in either the air-dry weight or the actual dry weight. It follows, then, that, per unit of fresh volume, the plants anchored contain a larger proportion of dry matter than those rooted in soil. On the other hand, the plants rooted in soil contain a larger proportion of all the constituents determined except starch, the anchored plants having a very marked increase in the proportion of this constituent. The difference in dry weight, then, is attributable to the larger starch content of the suspended plants.

Since the plants rooted in soil have a larger proportion of ash than those anchored, it seems evident that the retarded growth of the anchored plants was due to insufficient mineral food rather than to inhibited photosynthesis. The composition of the ash as determined in terms of dry weight is as follows:

|  | Plants rooted in soil. | Plants anchored. |
|---|---|---|
| Lime (CaO) ..........................................................per cent.. | 1.73 | 2.18 |
| Magnesium oxide (MgO)........................................do.... | .73 | 1.15 |
| Phosphoric acid ($P_2O_5$) ...........................................do.... | .56 | .31 |
| Potash ($K_2O$)...........................................................do.... | 7.97 | 6.40 |

It will be noticed that the anchored plants have a smaller proportion of potash and phosphoric acid. This, together with the diminished proportion of protein, strongly indicates that a sufficient supply of nitrogen, potash, and phosphoric acid was not appropriated by the anchored plants.

It can not be safely concluded from these results that the lake water does not contain nitrogen, potash, and phosphoric acid in sufficient proportion for the plants anchored in it to make a normal growth. It can be said, however, that either these constituents are not present in the lake water in sufficient proportion, or, if they are, the condition of the plants anchored in the lake water is such that these constituents can not be absorbed by the plant in proper proportions. This question will be considered further in connection with other data.

## CORRELATION OF GROWTH AND UNCONSUMED STARCH.

Early in this study of growth under varying conditions of nutrition it was discovered that per unit of fresh volume the plants of most growth yielded a smaller dry weight than those retarded in growth by reason of unfavorable conditions. Microscopic examination revealed the fact that the thrifty plants contained comparatively little starch, while those retarded in growth were literally gorged with it. A similar examination at the conclusion of each experiment showed that whether in the aquarium experiments or in those with nutrient solutions, the starch had accumulated in the plants in proportion as growth had been retarded; so it may be said that so far as these experiments are concerned the amount of unconsumed starch in the tissues of the plant varies inversely with the growth. We have thus from direct microscopic examination, as well as from chemical analysis, evidence that the retarded growth in these cases can not be attributed to conditions unfavorable to the photosynthetic process.

## LIGHT AND MECHANICAL CONTACT AS FACTORS IN THE DEVELOPMENT OF LATERAL ROOTS.

### RANUNCULUS AQUATILIS TRICHOPHYLLUS.

It has been noted that when fragments of this plant are left floating, the new roots arising at the nodes grow directly downward and do not branch until entering a substratum.

Three conditions suggest themselves as possible factors in determining the development of these lateral roots. The first is light; the second, mechanical contact as a stimulus; the third, a difference of osmotic strength between the solution in the soil and that above it. The last supposition is very improbable, as lateral roots develop abundantly in clean washed sand, and in this case the roots are probably exposed to a solution whose osmotic strength is the same as that of the solution above the sand. The following test was made with a view of ascertaining the determining factor:

*Experiment No. 17.*—This experiment was conducted in the greenhouse from April 10 to May 18. Fresh cuttings, 25 cm. in length, were mounted in 10-ounce bottles, submerged in tap water contained in cylindrical battery jars. A loose cotton stopper wrapped about the cutting kept it in proper position, and did not prevent the diffusion of water inside the bottle with that outside. Four conditions entered into the test: Bottles wrapped in black cloth to exclude light; bottles not covered, roots being exposed to light; bottles not covered, but containing sand, and bottles not covered, but containing granulated glass.

About three nodes of the cutting were inside the bottle in each case, and when first mounted the cuttings were without roots. Five cuttings were in conditions 1 and 2, and 3 cuttings in conditions 4 and 5. In No. 4 the intention was to have mechanical contact with a transparent substratum, but a layer of glass deep enough for a substratum greatly reduces the light. In no case did roots from nodes above the stopper have lateral branches, and, as the number of these roots was practically equal to the number arising from nodes below the stopper, only the latter are given in the table.

*Influence of light on the development of root system of Ranunculus aquatilis trichophyllus.*

| Condition. | No. of main roots. | Total length of main roots. | Average length of main roots. | No. of lateral roots. | No. of lateral roots per main root. | Total stem length. |
|---|---|---|---|---|---|---|
| | | Cm. | Cm. | | | Cm. |
| Wrapped bottles | 22 | 1,048 | 47.63 | 73 | 3.8 | 191 |
| Unwrapped bottles | 28 | 459 | 16.4 | 0 | 0 | 170 |
| Sand substratum | 15 | 262 | 17.46 | 66 | 4.4 | .......... |
| Glass substratum | 16 | 296 | 18.5 | 13 | .81 | .......... |

These figures show (1) that, other conditions being equal, light inhibits the formation of lateral roots and retards the growth of main roots; (2) that plants with roots in wrapped bottles and consequently a more extensive root system do not make a proportionately greater growth in stem length. The remaining figures are of little value as they stand.

## RECAPITULATION AND THEORETICAL DISCUSSION.

Seven species of frequent occurrence in our aquatic flora have been submitted to a direct test to- determine the influence of a soil substratum upon their growth. In five of these cases the actual growth in length has been measured. All of the seven species grow naturally rooted in the substratum. *Chara* has only rhizoids, of course, but the others have roots, and, with the exception of *Myriophyllum*, root-hairs also. Not one of these plants can make an optimum growth in tap water if the roots are prevented from entering the substratum. If allowed to root in clean-washed sand a better growth is obtained, but not nearly so good as when the roots freely penetrate a good soil. The difference in amount of growth between plants rooted in sand and those in soil, in terms of the former, was for *Potamogeton*, 480.36 per cent; for *Elodea*, 340.57 per cent; for *Ranunculus*, 62.96 per cent; for *Myriophyllum*, 46.43 per cent; for *Chara*, 44.58. No reason is apparent for not considering these figures as indicating the relative dependence of the different species upon the soil. This is, however, a secondary matter as compared with the fact, herein demonstrated, that a soil substratum is requisite for normal growth. The root-development of the anchored plants is undoubtedly retarded by exposure to light, but, as is shown in experiment No. 17 (p. 515), with *Ranunculus*, the more extensive root-system is not accompanied by a correspondingly greater growth in stem length. Why the plants rooted in sand should do so much better than those anchored above sand is not altogether certain, but the more extensive root-system which develops in sand will account for a part of the difference. That a sand substratum 10 or 15 cm. deep should concentrate the salts of the supernatant water to a degree sufficient to influence the amount of growth is hardly probable. In each case the sand was thoroughly clean when the experiment began, but some undissolved substance may have become embedded in it during the experiment, although the water in the aquarium was frequently stirred and siphoned out.

In experiment No. 10 (p. 504) sandy, clayey, and loamy soils were compared with respect to the suitability of each, and it appears that *Vallisneria* and *Chara* make a better growth on a good loam soil, just as many land plants do.

The experiments with Sachs's solution show that the plants can not make as good a growth in it, either with or without a substratum, as in soil and tap water. These experiments are not as extensive as they should be and must be regarded as indicating rather than establishing conclusions. What is the most suitable solution for those plants and whether they will make an optimum growth in any solution unless rooted in a substratum must be left as open questions. The suitability of Sachs's solution for many land plants is well known, and why these aquatics should be unable to grow in it can, so far as these experi-

ments have gone, be only surmised. It is noteworthy that in the absence of a substratum Sachs's solution totally inhibits root formation in the case of *Elodea;* with *Potamogeton* a very few roots appear, but shortly die; with *Ranunculus* more roots appear, but they reach a length of only a few centimeters. All of these plants will develop roots better in Sachs's solution if allowed to send them into a sand substratum, but even here the development is much less than with plants anchored in tap water without a substratum. It is evident that the sand substratum, as well as the solution and light, is a factor influencing root development, unless we assume that the sand changes the strength or quality of that part of the solution which saturates it. True and Oglevee (1904) found that the presence of insoluble substances, such as sand, paraffin, and filter paper, in solutions "exerts an effect closely paralleling that of simple dilution." As the Sachs's solution was frequently renewed we can not suppose that the quality of the solution gradually became unfavorable during the experiment. In experiment No. 13 (p. 508), where *Ranunculus* is grown in Sachs's solution without a substratum, with a sand substratum, and with a soil substratum, we see that the soil here is a very important factor. The average length in the three groups was 19.16 cm., 18.8 cm., and 26 cm., respectively. This brings out the interesting fact that the soil in some way helps the plant under otherwise unfavorable conditions.

That the roots of most of our common aquatics are provided with root hairs is significant, and certainly indicates that absorption is an important function of the roots. It is interesting to note in this connection that of the two species found to be least dependent upon the soil, one is *Chara*, an alga with only rhizoids instead of roots, and the other *Myriophyllum*, which has roots, but not root hairs.

Experiments 14 and 15 (pp. 510 and 511) demonstrate that the roots will absorb lithium nitrate and that the salt is carried upward into the stem and leaves. Reference to the experiment will show that diffusion will not account for this result and there is no escape from the conclusion that an upward current carries the salt to the leaves.

Experiment 16 (p. 511) demonstrates the absorption of a given amount of tap water in a given time by the roots developed from cuttings suspended in tap water. It was not intended here to determine the rate of absorption, but only to demonstrate the fact. It is to be regretted that the several attempts made to measure the exudation from the stem and leaves were unsuccessful. Hochreutiner's efforts to do the same thing were not rewarded by results because of the difficulties encountered in the technique. If, however, a large absorption is a fact, the exudation is a necessary consequence. Whether this exudation is in any way comparable to the transpiration of terrestrial plants is an interesting and relevant question, but the answer to such an inquiry is not considered possible on the basis of these experiments.

The chemical analysis of *Vallisneria* shows that the metabolism of plants denied a substratum is very different from that of plants allowed to root in the soil. The former show a marked excess of calcium and magnesium, while the latter contain a larger proportion of protein, potassium, and phosphorus. This change of metabolism manifests itself outwardly by a greatly retarded growth, and microscopic examination reveals that an abnormal amount of starch has accumulated in the tissues. This accumulation of starch is so great that the dry weight of a given fresh volume is considerably more than is obtained from an equal fresh volume of plants grown rooted in the soil.

The last experiment, No. 17, with *Ranunculus*, shows that light is the factor which prevents the formation of lateral roots and which also retards the growth of the main root. However, the more elaborate root system which develops in the dark does not aid the plant to make a proportionately greater growth when the roots are not allowed to enter the soil.

From the aquarium experiments it is evident that these attached aquatics are dependent upon the soil for optimum growth. Not one of the species investigated, except possibly *Chara*,[a] can survive the growing season unless rooted in the soil, and even *Chara* does not make an optimum growth under any other conditions.

While the aquarium experiments establish the fact as stated, they do not furnish adequate explanation of the fact. It may first be asked: Does the soil furnish plants rooted in it with substances that are not available for plants suspended in the water over it?

Concerning this question we may consider, first, that soils have the property of withdrawing salts from solution. Way (1850) discovered that liquid manure filters through soil to a clear solution containing both organic and inorganic matter in diminished quantity. Liebig (1858, p. 109) and others took up the matter until this absorptive capacity of soils is well established. For a time authors were divided as to whether this fixation, or rather retention, of salts by the soil is a physical or chemical process, but the general agreement now is that both physical and chemical processes operate. (Kubel-Tiemann-Gärtner, 1889.) Pfeffer (1900, p. 166), summarizing from the various researches, states that most soils absorb the oxides, salts of the alkalies, and alkaline earths of potassium, ammonium, magnesium, sodium, and calcium in relative quantities in the order mentioned. It must be remembered, however, that this retention of dissolved substances by the soil is neither absolute nor permanent.

In the case of the lake there are probably operating two opposing

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[a] Davis (1901) states that culture experiments made by him demonstrated the fact that *Chara* takes its lime from the water and not from the soil. However this may be, it is certainly true that *Chara* makes its best and most vigorous growth when rooted in a good soil.

processes, in which the soil tends to withdraw salts from solution, and the water tends to bring salts of the soil into solution. Excluding other factors, these two processes would probably establish an equilibrium resulting in a constant concentration. But. plants, and especially those attached to the soil, are important factors in the redistri-. bution of matter, which is constantly going on. The roots in respiration excrete carbon dioxide, which helps to bring otherwise insoluble salts into solution.

Apparently the substances needed by the plants are the ones most firmly retained by the soil, and yet it can not be said that the water does not contain enough of these salts for the larger plants. That plants have a quantitative selective power is certain, and their capacity for concentrating salts from very dilute solutions is well established, especially in the case of potassium in land plants and of iodine in some marine forms. Liebig (1858, p. 140) found that the ash of *Lemna* contained of potassium 13.16 per cent and of phosphoric acid 8.73 per cent, while the inorganic residue from the water in which the *Lemna* was growing contained these substances in the respective proportions of 3.97 per cent and 2.619 per cent. As *Lemna* and *Ceratophyllum* must derive their mineral nourishment exclusively from the water, it is evident that the necessary salts are present, and in sufficient quantity for some plants.

Granted, then, that the necessary salts are present, though in very small quantity in some cases, it may next be asked: Are the salts present in suitable proportion? The evidence at hand hardly furnishes satisfactory reply. Chemical analysis of *Vallisneria* indicates that they are not. The marked excess of calcium and magnesium in the anchored plants is a noteworthy fact. According to Loew's (1901, p. 16) hypothesis, calcium is especially required for the formation of nucleoproteids and magnesium for facilitating the assimilation of phosphoric acid. Should the excess of lime be too great, the magnesium is displaced and the phosphoric acid, combining with the lime, becomes insoluble. The result (Loew, 1901) is the same as if the supply of phosphoric acid were too limited, and the plant succumbs to starvation. Loew's hypothesis is hardly applicable to my results, however, as the ratio of magnesium to calcium in plants rooted in soil is about the same as that in the anchored plants.

The accumulation of starch in the anchored plants is the most positive evidence of abnormal metabolism revealed by the chemical analysis, and this, in connection with the retarded growth, furnishes a basis for further investigation. Is the growth retarded because the starch is formed too rapidly, or does the starch accumulate because growth is retarded? Pfeffer (1900, p. 515) states that "the mobilization of reserve food materials is regulated by the amount consumed;" also (p. 425), "when growth is inhibited the consumption, and hence also

the translocation, of carbohydrates ceases, so that if the assimilation of carbon dioxid is possible, the assimilatory products will accumulate in the leaves until the inhibitory limit is reached, and this result will be produced whether the stoppage of growth is due to a deficiency of potassium or phosphorus, or to widely different causes." From this point of view the accumulation of starch is a consequence and not a primary cause of retarded growth.

Proteid synthesis is the other very important metabolic process, and the chemical analysis does suggest some interference with this function. The diminished quantity of potassium and phosphorus may mean that the plants could not assimilate these elements rapidly enough to furnish proteids for new tissue. (Pfeffer, 1900, p. 430.) With proteid synthesis once retarded pathological conditions would soon arise; non-diosmosing substances might be formed which would still further interfere with normal metabolism; the activity of enzymes might be inhibited, thus favoring starch accumulation—in fact, we might make several suppositions, all of which would be more or less directly associated with inhibited proteid synthesis. On the other hand, starch formation itself requires proteids for the plastids; but it is not known what may be the capacity for photosynthesis of the plastids already present before abnormal conditions arise.

Further, it may be asked: Is a uniform environment unfavorable to the plant? When the roots are in contact with the substratum a possibly much better opportunity is afforded for exercising a quantitative selective power than when they are merely hanging in a solution identical with that which surrounds the remainder of the plant. Perhaps this diversity of environment means much to the plant by way of favoring the excretion of waste products as well as securing larger quantities of certain salts.

This leads to the final inquiry: Is the function of absorption localized? The plants which naturally live independently of a substratum have a much simpler structure than those like *Ranunculus* or *Potamogeton*, and it is quite possible that in the latter cases the functions of absorption and excretion are so localized that the plant can not continue normal metabolism when bathed over its entire surface with one nutrient solution, even though the solution contain all the necessary ingredients in suitable proportion and chemical combination. Possibly one benefit of a substratum is to furnish the roots with a solution which is not isotonic with that which bathes the leaves, although isotonic must be considered here as applying to each salt individually, as the plants have a varying capacity for absorbing and incorporating different salts.

The presence of root hairs may be regarded almost as prima facie evidence that the roots bearing them are organs of absorption. That root hairs are absent in some few species is not evidence that they are

unnecessary structures for water plants in general, for there are terrestrial plants (Schwarz, 1881–1885, p. 168) whose roots do not develop root hairs.

The necessity for going further into the chemistry of plant metabolism is apparent, and we can only say that when these plants are denied a substratum of soil the normal processes of metabolism are altered to a fatal degree.

### CONCLUSIONS.

1. *Vallisneria spiralis, Ranunculus aquatilis trichophyllus, Elodea canadensis, Myriophyllum spicatum, Potamogeton obtusifolius,* and *P. perfoliatus* are dependent upon their rooting in the soil for optimum growth, and can not survive a single season if denied a substratum of soil.

2. The roots of these plants are organs of absorption as well as of attachment.

3. There is an upward current in these plants, from roots to stem and leaves.

4. When these plants are denied a substratum, pathological conditions arise which are manifested by an accumulation of starch and a retarded growth with subsequent death.

5. The retarded growth of plants denied a substratum is not due to inhibited photosynthesis.

6. The plants anchored over a soil substratum do not have a more favorable environment than those anchored over a clean washed sand substratum.

7. Many of the plants rooting in soil develop root hairs, and the presence of these structures is the rule rather than the exception.

8. In the case of *Ranunculus aquatilis trichophyllus* light inhibits the formation of lateral roots.

9. *Ceratophyllum* and some other floating plants are able to absorb their nutrient salts directly from the surrounding water.

From the results of this investigation the following deductions are considered probable:

1. The above conclusions are applicable to all aquatic plants which grow rooted in a soil substratum, and especially to those whose roots are provided with root hairs.

2. The primary cause of the retarded growth of anchored plants is their inability to secure enough phosphorus and potassium, and possibly other elements.

3. When proteid synthesis is inhibited by an insufficiency of phosphorus and potassium, pathological conditions arise which permit the accumulation of starch.

4. These plants are terrestrial forms adapted to an aquatic habit rather than descendants of plants in which the functions of absorption and excretion are not localized.

5. These rooted aquatics are important contributors to the plankton food supply, because when living they organize matter that may be used as food and in death they yield important salts and organic substances to the water. Artari (1901) finds that certain algæ prefer organic nourishment, and it is quite possible that many of the forms so abundant on wounded and decaying portions of the larger plants derive considerable nourishment therefrom.

### ECONOMIC SIGNIFICANCE OF RESULTS.

The foregoing investigation may be regarded as a step in the endeavor to ascertain those factors which determine the quantity of food fish occurring in the Great Lakes. From the introduction it appears that the larger plants are already credited with favoring the increase of fish food by protecting the bottom soil against wave action, and by affording a shelter for many small animals and young fish, as well as by acting as mechanical supports for the algæ, which are used as food by many animals. If the observations recorded in this paper are correct, there must now be definitely assigned to the rooted aquatic plants a nutritive rôle of which they have hitherto been only suspected. The roots of the plants investigated are true absorbing organs, taking from the soil valuable salts that would otherwise be retained by it, and furnishing these salts to the growing stems and leaves for the building up of more plant tissue. So dependent upon the soil are these rooted aquatics that they can not survive a growing season if deprived of it. Thus, instead of taking their mineral food exclusively from the water, as formerly supposed, and so temporarily withdrawing valuable salts from the water, these rooted aquatics take their food from the soil and organize it into vegetable matter. Upon the decay of the vegetable matter this food material is believed to pass into solution in the water. It should there nourish the plankton algæ, which, in their turn, are used as food by the smaller animal forms, and these in turn are fed upon by larger animals and by fishes.

In western Lake Erie, where large areas of the substratum in coves and bays are occupied by dense fields of plants (aquatic meadows), the changing winds often create currents which carry out into the lake large quantities of plant débris. This during the period of slow oxidation represents so much organized matter available for plankton nutrition, and in final decay yields important mineral salts to the water, thus adding to the food supply of the plant plankton.

That there is a direct relation between the quantity of food fish and the quantity of plankton has long been believed. Recently Kofoid (1903) has produced quantitative evidence to show that in the Illinois River and its back waters such a relation exists, in the sense that "there is in general a correspondence between plankton production and the product of the fisheries, in that the direction of movement in

both is usually the same. They rise or fall together." The argument presented by Kofoid will not be critically discussed here.

In view of the results obtained in this investigation, it appears highly probable that through the mediation of the attached plants the abundant mineral salts held fixed by the soil become available for the nourishment of the phytoplankton. On this basis it is possible to attribute the scarcity of plankton and fish in some waters in part at least to the scarcity of the larger, rooted, aquatic plants. Kofoid (1903) shows, in the case of Flag Lake, that an abundant rooted vegetation is favorable to a high plankton production. In the other lakes examined by him he has made careful measurements of the plankton at frequent intervals for a period of five years, and he divides these lakes into two groups—vegetation rich, which contain an abundance of submerged aquatic plants, and vegetation poor, which contain but little submerged aquatic vegetation. He concludes from his measurements of the plankton that the vegetation-rich lakes produce less plankton than the vegetation-poor lakes. He says: "This relation of vegetation to plankton may be formulated as follows: The amount of plankton produced by bodies of fresh water is, other things being equal, in some inverse ratio proportional to the amount of its gross aquatic vegetation of the submerged sort." (Kofoid 1903, p. 484, footnote.) The relatively small amount of plankton in vegetation-rich lakes Kofoid attributes to a number of factors. In part it is due to the fact that the vegetation shuts out the heat and light of the sun and thus keeps all but the surface layer of water in shade and at low temperature, so that plankton algæ do not develop readily. In part it is probably to be attributed to the presence of plankton-eating animals which find shelter in the dense, gross vegetation. Chiefly, however, he attributes it to the fact that the larger aquatic plants take from the water and utilize in their growth the greater part of the available food materials. Thus plankton vegetation is unable to develop because the water has been depleted of the food substances necessary for its nutrition. Hence the development of an abundance of submerged aquatic vegetation results in a diminished plankton, while a scant submerged vegetation is correlated, other conditions being the same, with a more abundant plankton.

While Kofoid recognizes in the case of Flag Lake that an abundant rooted vegetation is favorable to plankton production, he points out that this vegetation is either succulent (*Sagittaria, Pontederia, Nymphæa, Nelumbo*), in which case it dies down and decays in early fall, or it is emergent (e. g., *Scirpus*), in which case it dies down and decays when broken down by ice and winter floods. The vegetation of Flag Lake is rooted, and Kofoid suggests that the richness of the lake in plankton is to be attributed to the food materials drawn from the soil by these rooted aquatic plants and dissolved in the water by

their decay. So far as concerns vegetation like this, his conclusions are precisely those which seem necessarily to follow from the experimental results recorded in this paper.

In contrast to Flag Lake, however, are Dog-fish and Quiver lakes, which are filled with a rich growth of submerged vegetation. This "consists in the main of *Ceratophyllum*, with an admixture of *Elodea* and *Potamogeton* toward the margin" (Kofoid, 1903, p. 244). It is this sort of submerged non-rooted vegetation which Kofoid shows to be unfavorable to an abundant plankton, so that lakes which contain it and which he calls vegetation-rich have less plankton than otherwise similar lakes which are without it. The conclusion that an abundance of submerged vegetation is inimical to the development of a rich plankton seems at first sight to be at variance with the conclusions reached in this paper, and Kofoid's general formula, "The amount of plankton produced by bodies of fresh water is, other things being equal, in some inverse ratio proportional to the amount of its gross aquatic vegetation of the submerged sort," is certainly not in accordance with these conclusions. Yet the apparent contradiction between his results and those here recorded disappears when it is remembered that the submerged vegetation to which he has reference is composed chiefly of *Ceratophyllum*, and that *Ceratophyllum* is a rootless form, which undoubtedly draws its food supply from the water only. It thus competes with the phytoplankton for food, and an abundant growth of it is necessarily correlated with a scant growth of phytoplankton. On the other hand, the submerged vegetation considered in this paper is rooted; it draws its mineral nourishment from the soil and in decay yields it to the water. It does not, therefore, compete with the phytoplankton, and its presence is, from a nutritive standpoint, favorable to the development of phytoplankton.

From the standpoint of nutritive relations, then, all vegetation of fresh waters may be divided into two classes: (1) The rooted vegetation, which may be either emergent (e. g., *Scirpus*) or submerged (e. g., *Vallisneria*, etc.), and which includes nearly all the gross aquatic plants. Of these it may be said that they draw their mineral food from the soil and are thus favorable to the growth of the phytoplankton. (2) Nonrooted vegetation, consisting of (a) gross, nonrooted phanerogams, made up almost wholly in temperate regions of *Ceratophyllum* and the *Lemnaceæ*, and (b) minute, nonrooted cryptogams, which are mostly members of the phytoplankton. All these nonrooted plants draw their mineral food from the water, and hence the two subdivisions, the gross and the microscopic, compete with one another, so that an abundance of nonrooted gross plants results in a reduced plankton. Kofoid's formula modified to bring it into accord with all the facts would read, "The amount of plankton produced by bodies of fresh water is, other things being equal, in some inverse ratio proportional to the amount of its gross nonrooted vegetation and in some

direct ratio proportional to the amount of its gross rooted vegetation." In the final paragraph of that part of his paper which deals with this subject, Kofoid (1903, p. 502) recognizes that the distinction should be drawn between rooted and nonrooted vegetation, and suggests that experimental proof is desirable for the generalization which he advances. Such experimental proof I had already offered (Pond, 1901) in a preliminary note, to which Kofoid does not refer, though he refers to Pieters (1901, p. 73, footnote), in which this note is cited. (See also Pond, 1902, p. 89.)

If we accept the conclusions reached in this paper that gross rooted vegetation is favorable to plankton production, and if we further accept the current argument that fish production is dependent on plankton production, the practical application of the results of this investigation are simple. In the stocking of ponds for fish culture care should be taken to have a good soil for the bottom; not a stiff clay nor sand, but a good loamy soil, such as is favorable for land plants. The species allowed to grow should be those which are known to possess roots and to be very dependent upon the soil, such as *Vallisneria spiralis*, the so-called eelgrass, and *Potamogeton*, or pond weeds; not forms without roots, such as *Ceratophyllum*, or those less dependent upon the soil. In natural lakes choked with a growth of *Ceratophyllum*, the removal of this form and the substitution for it of rooted plants offer possible means of increasing the supply of edible fish.

The poverty of the Great Lakes in plankton may be attributed to several causes. One of these is, doubtless, the relatively small shore area in these waters occupied by rooted aquatics. The comparatively short shore line, the narrowness of the shore area, and the mechanical action of the waves, all tend to limit the growth of rooted plants, hence to limit the productive capacity of the lake in plankton and, according to the current belief, in fishes.

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